

<p align="center">8 DETECTION OF BLOOD</p>	<p align="center">Page 1 of 2</p>
<p align="center">Division of Forensic Science</p> <p align="center">BLOODSTAIN PROCEDURES MANUAL</p>	<p align="center">Issue No: 1</p>
	<p align="center">Effective Date: 15-October-2004</p>
<p align="center">8 DETECTION OF BLOOD</p> <p>8.1 Combined Phenolphthalein-Tetramethylbenzidine (PTMB) Test (References Forensic Biology Procedure Guide)</p> <p>8.1.1 Safety Considerations</p> <p>8.1.1.1 Phenolphthalin - Caution! Avoid contact and inhalation!</p> <p>8.1.1.2 Potassium hydroxide - Caution! Corrosive! Poisonous!</p> <p>8.1.1.3 Tetramethylbenzidine - Caution! Harmful if swallowed, inhaled or absorbed through skin! Emits toxic fumes under fire conditions!</p> <p>8.1.1.4 Glacial acetic acid - Caution! Corrosive! Flammable!</p> <p>8.1.1.5 Ethanol - Caution! Flammable! Poisonous!</p> <p>8.1.1.6 Oxidized zinc - Caution! Danger of spontaneous combustion if allowed to dry!</p> <p>8.1.2 Materials and Equipment</p> <p>8.1.2.1 Dropper bottles</p> <p>8.1.2.2 Cotton swabs</p> <p>8.1.3 Stock Solutions</p> <p>8.1.3.1 Phenolphthalin Stock Solution (Obtain from Forensic Biology)</p> <p>8.1.3.2 Tetramethylbenzidine (TMB) Stock Solution (from Forensic Biology)</p> <p>8.1.4 Working Solutions (From Forensic Biology)</p> <ul style="list-style-type: none"> • Distilled water • Ethanol • 3% Hydrogen peroxide • 1:5 dilution of phenolphthalin stock solution in distilled water (1 part of the phenolphthalin stock solution diluted with 4 parts of distilled water) • TMB stock solution <p>8.1.5 Minimum Standards and Controls</p> <p>8.1.5.1 On the day of use a positive reagent control (known bloodstain) and a negative reagent control (distilled water) must be tested to ensure that the reagents are working properly. The results of this testing must be documented in the case file.</p> <p>8.1.5.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.</p>	

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<p>8.1.5.3 If the results of the test are positive, a substrate control (when available) must also be tested, unless the stain is on a cotton swab, and the results of the testing documented in the case file. It is not necessary to test submitted control swabs.</p> <p>8.1.6 COMBINED PHENOLPHTHALEIN-TETRAMETHYLBENZIDINE (PTMB) TEST PROCEDURE</p> <p>8.1.6.1 Gently rub a suspected stain with a cotton swab which has been moistened with distilled water or place a small cutting of the stain in a small test tube or microtiter plate, or on filter paper and moisten with distilled water if desired.</p> <p>8.1.6.2 Add one drop of ethanol.</p> <p>8.1.6.3 Add one drop of 1:5 dilution of phenolphthalin (i.e., the working solution of phenolphthalin).</p> <p>8.1.6.4 Add one drop of 3% hydrogen peroxide.</p> <p>8.1.6.5 Note any color change. An immediate pink color is expected if blood is present.</p> <p>8.1.6.6 Add one drop of tetramethylbenzidine stock solution.</p> <p>8.1.6.7 Note any color change. An immediate blue-green color is expected if blood is present.</p> <p>8.1.6.8 Interpretation</p> <p>8.1.6.8.1 Positive Reaction = Immediate pink color at 1.1.6.5, followed immediate blue-green color at 1.1.6.7</p> <p>8.1.6.8.2 Negative Reaction = No color change at 1.1.6.5, followed by no color change at 1.1.6.7</p> <p>8.1.6.8.3 Inconclusive Reaction = Development of color combinations other than those specified for a positive reaction, including one test positive and the other test negative</p> <p>8.1.6.9 Reporting Results</p> <p>8.1.6.9.1 Report positive test results as “the results of chemical tests <u>indicate</u> the presence of blood” or use similar wording.</p> <p>8.1.6.9.2 Report negative test results as “no blood was <u>detected</u>” or use similar wording.</p> <p>8.1.6.9.3 Report inconclusive test results as “the results of chemical tests for the presence of blood were inconclusive” or use similar wording.</p> <p align="right">◆END</p>	